

(62.nC/ml of tritium) plus a known amount of unlabelled estriol. The concentration in the first column recites only the known unlabelled estriol concentration.

TABLE II

Conc. Unlabelled Estriol ng/ml	Number of Cycles	Average Percent Bound**	Standard Deviation
0	13	84.8	0.57
0*	22	84.6	1.02
.27	5	77.1	1.1
.54	14	70.6	2.2
1.08	10	57.3	1.1
2.7	13	36.1	1.9
5.4	8	24.3	.47

*These two series were separated in time.

**The bound fraction was measured after release by the regenerating solvent.

All runs were made on a single antibody mass. Twenty one actual clinical samples were run through the same mass, interspersed among the runs reported in Table II. Additionally, several hundred more standards and other samples were run through the same antibody mass so that it has actually been regenerated about 500 times without detrimental loss in antigen binding efficiency. The tests reported in connection with Table II were all run on the operating prototype hereinafter described in Example III.

A variety of regenerating solvents have been tested and found satisfactory for release of bound antigen and regeneration of antibody. Some solvents, particularly ethyl and isopropyl alcohols, have demonstrated almost indefinite regeneration capacity. In fact, as noted with Solution B, an antibody mass has been regenerated about 500 times. The required characteristics of the solvent are that it breaks the antibody-antigen bond without destroying the flow characteristics or antigen binding efficiency of the antibody. In practice it will be necessary empirically to determine the proper solvent for any given system.

EXAMPLE III

A prototype system was constructed which employed a turntable sampler holding a plurality of samples and having a lifter for moving the sample tube 21 into and out of samples as they are sequentially rotated into position by a timer. A cyclic timer 41 was employed. A commercially available rotary sample valve was used to perform the functions of valves 22, 24 and 33 in response to signals from the timer. The valves 27 and 31 were simple twoposition electrically operated valves. Suitable metering pumps 28, 29 and 32 were used. Flow lines were small bore Teflon plastic tubes typically of 0.0012 to 0.0062 inch I.D. The contact chamber consisted of a polypropylene tube 0.125 inch inside diameter by 0.188 inch long. As used, the columns contained 0.04 ml of a Sephadex G-25 suspension at a concentration of 20 mg Sephadex/ml of a solution of 0.02M sodium phosphate at pH 7.5, 0.05 M NaCl, 0.01% merthiolate and 0.02% sodium oxide. Antibody to the antigen was covalently bonded to the Sephadex. The sample loop 23 had a capacity of 0.2 ml. Flow rates were:

Through the sample loop —0.45 ml/minute for 2 minutes

From tank 11 or 12 via pump 32 —0.112 ml/minute

From tank 13 via pump 39 —1.0 ml/minute.

The coil forming the detector cell has a volume capacity of 2.2 ml. The mixer 17 has a capacity of approximately 1 ml. Detection and counting was accomplished by means of a beta scintillation detector adjacent the coil and coupled to a counter not shown but which is a conveniently available type.

The immunoabsorbent in the contact chamber was an antibody coupled to Sephadex beads which were in turn immobilized in the contact chamber by nylon screen (325–400 mesh). Sephadex is a cross-linked dextran made by Pharmacia A.B. of Uppsala, Sweden. Such beads or other particulate solids with the ability to hold antibodies by co-valent bonds make convenient supports on which to bind the antibodies for immobilization.

As previously mentioned the tests reported in connection with Table II were all run in apparatus of the foregoing type.

In operation, a typical total cycle time for some tests was 28 minutes. This total included a three minute rinse cycle during which rinse from tank 11 flushed the system free from regeneration solvent. The sample loop, which does not require preliminary rinse, is at the same time flushed and filled with sample. The first three minute period is followed by a ten minute period during which reagent from tank 11 flows through the sample loop displacing sample therefrom into and through the contact chamber, the mixer and the detector. All of this is followed by a 15 minute regeneration period during which solvent from tank 12, by-passing the sample loop as previously described, flows through the contact chamber to release bound antigens thereby regenerating the immunoabsorbent. The released antigens are subjected to detection as they move through the flow cell to eventual discard.

In other tests, the cycle time has been reduced to less than fifteen minutes. In general, the cycle time can be reduced by increasing the concentration of radioactive tracer.

As noted, a suitable reagent for regeneration of the immunoabsorbent is one which will break the bond between the antigen and antibody but does not adversely affect the antibody. That is, it does not loosen it from its support nor reduce either its permeability or affinity for antigen. So far identification of suitable reagents has been empirical on the basis of behavior. However, once a suitable reagent for a given antibody-antigen system has been identified, it becomes a permanent reagent for that system.

Thus, the unexpected discovery that regeneration is possible and the identification of suitable reagents makes possible rapid analysis at greatly reduced costs. Moreover, the system is capable of automation thereby reducing human error with a concomitant increase in accuracy.

Throughout the specification reference has been made to bound antigen. This refers to the antigen bound to the antibody mass. The bound antigen is measured only after it has been released from the antibody by the regenerating solvent. If the percent recovery of bound (released) antigen is added to the percent of free antigen measured in any cycle, the total is consistently at 100% for practical purposes. This is significant because it confirms that substantially all bound antigen is released from the antibody and that the antibody is completely regenerated.